

COMMENTARY

What makes the α_{1A} -adrenoceptor gene express the α_{1L} -adrenoceptor functional phenotype?

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The α_{1A} -adrenoceptor is therapeutically exploited because of its prevalence in the lower urinary tract. The pharmacology shown by this lower urinary tract α_{1A} -adrenoceptor is different from that shown by other α_{1A} -adrenoceptors, which has led to it being subclassified as an α_{1L} -adrenoceptor. Only in the last few years was it shown that this pharmacologically distinct α_{1L} -adrenoceptor is a product of the α_{1A} -adrenoceptor gene. In this issue of the *BJP*, Nishimune *et al.* review the literature on α_{1L} -adrenoceptor pharmacology and discuss the possible molecular mechanisms by which the α_{1A} -adrenoceptor gene is able to produce two pharmacologically distinct adrenoceptor subtypes. Based primarily from their own research using cell lines transfected with α_{1A} -adrenoceptors, they conclude that a protein that interacts with the receptor is the most plausible explanation. The challenge remains to identify any such interacting protein and show how it is able to change the pharmacology of the receptor for different ligands.

LINKED ARTICLE

This article is a commentary on Nishimune *et al.*, pp. 1226–1234 of this issue. To view this paper visit <http://dx.doi.org/10.1111/j.1476-5381.2011.01591.x>

Abbreviations

BPH, benign prostatic hyperplasia; BRET, bioluminescence resonance energy transfer; CRELD, cysteine-rich epidermal growth factor-like domain; FRET, fluorescence resonance energy transfer

The most effective and rapidly acting pharmacological treatments for benign prostatic hyperplasia (BPH) are the α_{1A} -adrenoceptor antagonists, such as tamsulosin and alfuzosin (Miano *et al.*, 2008; receptor nomenclature follows Alexander *et al.*, 2011). This class of BPH therapeutic agents makes over US\$ 3 billion in worldwide sales (Ventura *et al.*, 2011). Previously, non-selective α_1 -adrenoceptor antagonists such as prazosin, doxazosin and terazosin were widely used, but these have now been largely superseded by tamsulosin and alfuzosin because of their greater selectivity for the α_{1A} -adrenoceptor subtype over the α_{1B} and α_{1D} -adrenoceptor subtypes. The proportion of the α_{1A} -adrenoceptor subtype expressed in the smooth muscle stroma of the prostate gland is greater than the proportion expressed in vascular smooth muscle, leading to a lower incidence of troublesome vascular side effects such as weakness, fatigue, postural hypotension

and dizziness, which were commonplace with the use of the non-selective α_1 -adrenoceptor antagonists.

α_{1A} -Adrenoceptors are abundant in the male lower urinary tract, and α_{1A} -adrenoceptor antagonists are very effective in relieving lower urinary tract symptoms associated with urethral obstruction caused by prostate enlargement. Despite this, prostate and other lower urinary tract tissues, from all species, do not show typical α_{1A} -adrenoceptor pharmacology (Nishimune *et al.*, 2012). When used in functional isolated tissue experiments, isolated tissues from prostate gland, urethra and bladder, all exhibit a low affinity for prazosin when compared with other α_1 -adrenoceptor-expressing tissues. A corresponding change in affinity is not seen with tamsulosin. This pharmacological anomaly led to the postulate that a fourth α_1 -adrenoceptor existed, which was termed the α_{1L} -adrenoceptor.

Only recently has it been demonstrated with the use of genetically modified adrenoceptor knockout mice that the prostatic α_{1L} -adrenoceptor phenotype requires the expression of the α_{1A} -adrenoceptor gene (Gray *et al.*, 2008; Muramatsu *et al.*, 2008). The term α_{1L} -adrenoceptor is not currently recognized as an official nomenclature term. Rather, the latest edition of the Guide to Receptors and Channels states, 'Some tissues possess α_{1A} -adrenoceptors that display relatively low affinity in functional and binding assays for prazosin ($pK_i < 9$) that might represent different receptor states (termed α_{1L} -adrenoceptors)' (Alexander *et al.*, 2011). Further, investigation of this phenomenon is critical to developing a better treatment for BPH as it would seem that men suffering from urethral obstruction resulting from BPH would benefit more from a selective α_{1L} -adrenoceptor antagonist rather than the selective α_{1A} -adrenoceptor antagonists like tamsulosin, which are currently used and show no selectivity between α_{1A} and α_{1L} -adrenoceptors. At present, there are no antagonists showing higher affinity for α_{1L} over α_{1A} -adrenoceptors.

Early attempts to explain how the α_{1L} -adrenoceptor phenotype could arise from the α_{1A} -adrenoceptor gene concentrated on whether genetic polymorphisms or splice variants of this gene could give rise to the phenotype. However, α_{1A} -adrenoceptors generated by known polymorphisms and splice variants in cell culture models all showed similar pharmacological characteristics to that of the α_{1A} -adrenoceptor (Shibata *et al.*, 1996; Suzuki *et al.*, 2000; Ramsay *et al.*, 2004), providing evidence that α_{1A} -adrenoceptor polymorphisms and splice variants were not associated with generation of the α_{1L} -adrenoceptor phenotype.

Subsequently, a 'interacting protein' hypothesis to explain the generation of α_{1L} -adrenoceptors from α_{1A} -adrenoceptors has been postulated, following observations from radioligand binding studies. The basis for this hypothesis is that radioligand binding studies of lower urinary tract tissues are almost always carried out using membrane homogenates and yield ligand affinities that fit the pharmacological profile of α_{1A} -adrenoceptor pharmacology. This is despite the findings that isolated intact preparations of prostate, urethra and bladder tissue display α_{1L} -adrenoceptor pharmacology when they have been used in functional studies. In an earlier review, Nishimune *et al.*, (2010a) suggested that this discrepancy was caused by the homogenization process disrupting the cell membrane and thus separating α_{1A} -adrenoceptors from the putative 'interacting protein'. They hypothesized that only when the α_{1A} -adrenoceptor is bound to the interacting protein does it display α_{1L} -adrenoceptor pharmacology. This idea is supported by their earlier paper that demonstrated that radioligand binding studies of tissue segments from lower urinary tract tissues produced a α_{1L} -adrenoceptor ligand affinity profile, while crude homogenized membrane fractions from these tissues yielded a α_{1A} -adrenoceptor profile (Muramatsu *et al.*, 2005). The presumption is that the more intact tissue segments maintain a more complete membrane with little or no disruption to the α_{1A} -adrenoceptor – interacting protein complex.

This interacting protein theory is a plausible and logical explanation for the occurrence of α_{1L} -adrenoceptor pharmacology in lower urinary tract tissues expressing abundant α_{1A} -adrenoceptors. Indeed, the interaction of proteins also covers the possibility that a receptor heteromer may be the

cause of the changed pharmacology of α_{1A} -adrenoceptors in the lower urinary tract. However, it is arguable whether tissue homogenization would disrupt the cell membrane sufficiently to destroy protein–protein interactions at the molecular level. Nevertheless, Nishimune *et al.* (2010b) identified cysteine-rich epidermal growth factor-like domain 1 α (CRELD1 α) as a novel down-regulating protein and therefore a protein that interacts with the α_{1A} -adrenoceptor. CRELD1 α was identified using a yeast two-hybrid approach, with the entire open reading frame of the human α_{1A} -adrenoceptor gene used as bait (Nishimune *et al.*, 2010b). Subsequent transfection of cDNA for the α_{1A} -adrenoceptor gene alone into CHO cells yielded cells expressing α_{1A} -adrenoceptors with the typical α_{1A} -adrenoceptor pharmacological profile, as well as a low proportion of α_{1L} -adrenoceptor sites. The small number of α_{1L} -adrenoceptors was presumably due to endogenous CRELD1 α as knockdown of CRELD1 α enhanced the expression of α_{1A} -adrenoceptors while over-expression of CRELD1 α reduced α_{1A} -adrenoceptor expression (Nishimune *et al.*, 2010b). Following this, they were able to produce α_{1A} -adrenoceptor-enhanced and α_{1L} -adrenoceptor-dominant cell lines that were used in ligand binding, and functional agonist and antagonist profile studies. Results for α_{1A} -adrenoceptor-enhanced and α_{1L} -adrenoceptor-dominant CHO cells were in agreement with the published profiles for α_{1A} and α_{1L} -adrenoceptor phenotypes, respectively, as seen in intact tissues.

Although the evidence presented in this paper is persuasive (Nishimune *et al.*, 2010b), there are still questions to be answered before the story is truly convincing. For instance, CRELD1 α over-expression yielded α_{1L} -adrenoceptor dominant cells expressing a higher proportion of α_{1L} -adrenoceptors; however, this was because of a reduction in α_{1A} -adrenoceptor binding sites rather than their conversion to α_{1L} -adrenoceptors as would be expected if CRELD1 α were a true α_{1A} -adrenoceptor interacting protein. Consequently, the expression of α_{1L} -adrenoceptor binding sites does not appear to change regardless of CRELD1 α expression. Alternatively, this observation itself could be interpreted as the CRELD protein inhibiting radioligand binding in some way, perhaps by internalization of the α_{1A} -adrenoceptor rather than changing its binding affinity to that of the phenotype of the α_{1L} -adrenoceptor. Experiments using membrane-permeable and membrane-impermeable agonists and antagonists would go a long way towards a clearer understanding of these results.

Other observations from this research throw up questions that need answers. For instance, the efficacy of agonists seen in cells with different levels of CRELD1 α expression differs when their activity is compared in functional assays. This introduces the possibility of ligand-biased signalling, which needs to be addressed and could further confound progress in this area. Similar agonist efficacy differences have been observed in functional experiments with intact tissues expressing the different phenotypes. Furthermore, over-expression of CRELD1 α (α_{1L} -dominant) also seemed to introduce an element of irreversible antagonism to prazosin at low concentrations, when compared with CRELD1 α knockdown (α_{1A} -abundant) cells in functional assays.

The idea of a α_{1A} -adrenoceptor interacting protein is a logical and plausible argument to explain the transition from α_{1A} -adrenoceptor gene to the α_{1L} -adrenoceptor functional

phenotype which is abundant in male lower urinary tract tissue. CRELD1 α appears to go part of the way to fulfilling the criteria that one would expect of an interacting protein for this receptor, but further experimental challenges remain to strengthen its case. Such experiments might include radioligand binding of whole cells versus membrane preparations using α_{1A} -adrenoceptor-expressing CHO cells with and without simultaneous CRELD1 α transfection. This would show whether the α_{1A} -adrenoceptor – CRELD1 α complex can be disrupted during membrane homogenization, leading to a change in pharmacological profile. Furthermore, demonstration of a direct interaction of the α_{1A} -adrenoceptor and CRELD1 α using resonance energy transfer techniques (FRET/BRET) in transfected CHO cells or immunoprecipitation techniques in native tissue could provide significant support for this hypothesis.

Conflicts of interest

The author states no conflict of interest.

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